Review

Caries Research

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Secondary Caries in situ Models: A Systematic Review

Audrey C.C. Hollanders[a] Nicolien K. Kuper[a] Tamires T. Maske[b]
Marie-Charlotte D.N.J.M. Huysmans[a]

[a]Department of Dentistry, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, The Netherlands; [b]School of Dentistry, Federal University of Pelotas, Pelotas, Brazil

Keywords
In situ models · Recurrent caries · Secondary caries

Abstract
In situ caries research serves as a bridge between clinical research and laboratory studies. In this kind of research, volunteers wear a removable intraoral splint or prosthesis containing research samples. Many different in situ models exist to investigate secondary caries. This systematic review compared currently existing secondary caries models and their lesion progression per day values. ***Materials and Methods:*** Three databases (Medline, Embase, and Cochrane) were searched for relevant literature. Bias risk was assessed and model parameters and caries-related outcomes were extracted by 2 independent researchers. Where possible, caries-related outcomes were normalized by estimating lesion progression per day by dividing lesion depth extracted from microradiographic or microhardness data by the number of days the study lasted. ***Results:*** The literature search identified 335 articles. After eliminating duplicates and selection, 31 articles were included. The models differed greatly on factors such as sample location, presence of fluoride in the model, and analysis methods. Three main groups could be identified by sample placement; 68% of models placed samples palatally in the upper jaw, and the lower jaw model could be divided into the buccal (26%) and approximal (6%) areas. Average lesion progression in enamel next to composite was 4.3 ± 2.8 µm (range1.1–8.8 µm/day). ***Discussion:*** Studies conducted with palatal models showed caries progression rates 2–5 times higher than the estimated clinical progression rates. Lesion progression per day could be a useful tool for future comparison of models and establishing a standardized model.

Dental caries is the localized destruction of susceptible dental hard tissues by acidic byproducts from the bacterial fermentation of dietary carbohydrates [Fejerskov and Kidd, 2015]. Primary caries is the term used to describe caries lesions developing on intact, natural tooth surfaces, as opposed to secondary or recurrent caries, which develops next to an existing restoration [Fejerskov and Kidd, 2015]. Two regions have been described when considering the process of secondary caries; the surface lesion, which develops perpendicular to the tooth surface and can be considered a primary lesion developing next to a restoration, and the wall lesion, which develops perpendicular to the tooth/restoration interface (Fig. 1) [Hals and Nernaes, 1971]. Several study types are currently used to investigate secondary caries. These include in vi-

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E-Mail karger@karger.com
www.karger.com/cre

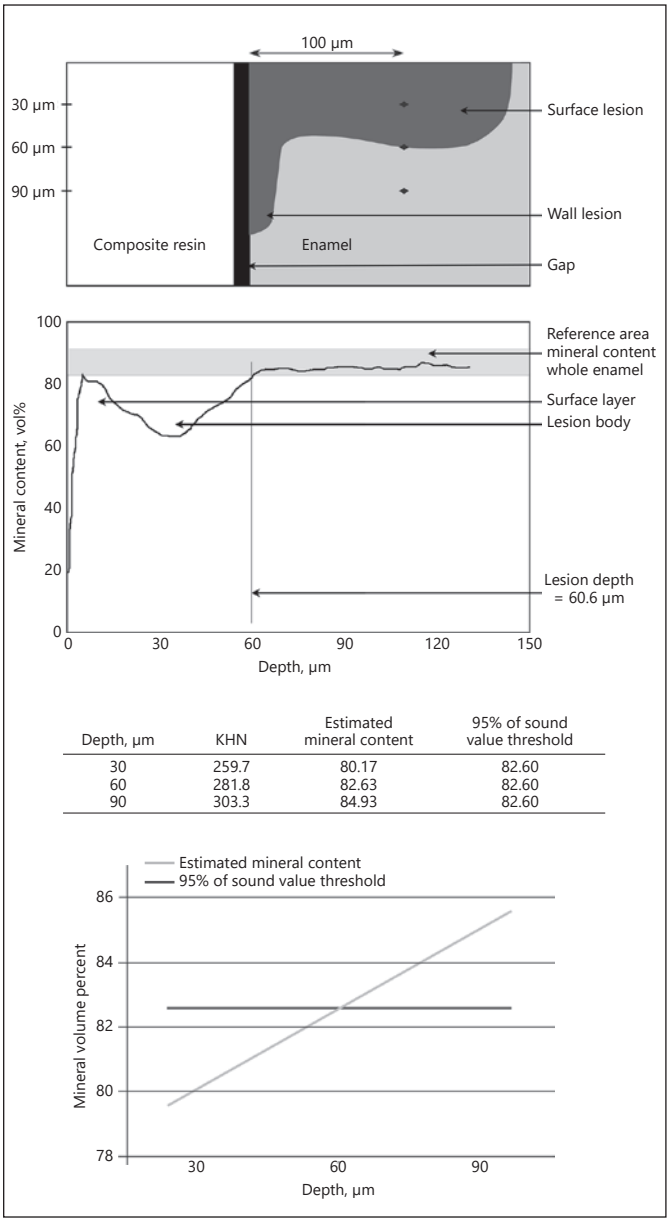
Audrey Cornelia Catharina Hollanders
Radboud University Medical Center
Prins Hendrikstraat 69
NL–6521AX Nijmegen (The Netherlands)
E-Mail audrey.hollanders@radboudumc.nl and audreyhollanders@gmail.com

tro, in situ, and in vivo models [Lynch and ten Cate, 2006; Bernardo et al., 2007; Kuper et al., 2015]. To simulate a failed interface between a tooth and a restoration, a gap can be formed between the two during restoration prior to cariogenic challenge.

In an attempt to approximate the clinical situation more closely than in an in vitro model for the purpose of caries research, in situ models are used to investigate secondary caries [Askar et al., 2017]. In in situ studies, a group of volunteers wears an appliance containing dental samples in their mouth. Research conditions can be tested on these samples. The experimental conditions in the various in situ models can differ greatly. Recently, a systematic review evaluated in situ studies in secondary caries [Askar et al., 2017]. It aimed to compare the performance of 7 classes of restorative material based on results from 9 in situ studies. The authors attempted to create a network model displaying the secondary caries susceptibility of several restorative materials. They found, however, that inconsistency was too great to draw clear conclusions. Our review aims to address the inconsistencies in such studies, in an attempt to increase the comparability of studies in the future.

The lesion progression per day of (secondary) caries lesions has been described in in situ studies and could be a valuable way to compare different models [Thomas et al., 2007]. However, the variations in analysis methods of secondary caries formation has led to differences in outcome measures [Kielbassa et al., 2003; Vasconcelos et al., 2014]. Microradiographic techniques are considered the gold standard in the measurement of caries lesions; they provide both lesion depth and mineral loss values [Thomas et al., 2006]. Transversal microradiography (TMR), a microradiographic technique, uses image analysis software to render a mineral concentration profile which is used to calculate lesion depth and integrated mineral loss. Cross-sectional microhardness is also frequently used, comprising microhardness measurements on a cross-section of the surface. As microhardness and mineral volume are related, it is possible to estimate one from the other [Featherstone et al., 1983; Kielbassa et al., 1999b].

Secondary caries lesions are often divided into 2 distinct types, surface and wall lesions (Fig. 1). This review will focus on comparing surface lesion development next to restorative materials, since wall lesion measurement is, as of yet, comparatively rare and has not been standardized. In some cases, wall lesions have been measured perpendicular to the interface [Thomas et al., 2007], but also parallel to the interface [Grossman and Matejka, 1995]. This leads to incomparable results.



Depth, µm	KHN	Estimated mineral content	95% of sound value threshold
30	259.7	80.17	82.60
60	281.8	82.63	82.60
90	303.3	84.93	82.60

Fig. 1. An example of the lesion depth calculation. The recorded lesion depth in this case was 60.6 µm [Chimello et al., 2008a]. KHN, Knoop hardness number; vol%, volume percent.

Clearly, protocols for in situ studies vary. Our aim was to systematically review in situ models for secondary caries, compare their methodology and parameters, and, where possible, determine a link to the reported lesion progression rate. In line with this, we hoped to come up with recommendations for improving in situ studies and ways to make them more comparable.

Materials and Methods

Inclusion Criteria

- The study should use a removable device containing samples that is worn intra-orally by volunteers.
- These samples should consist of dental tissue (enamel and/or dentin) and a restorative material.
- Dental caries should be allowed to develop while the samples are exposed to the in situ environment.
- The study should have a caries-related outcome, producing measurements such as lesion depth, mineral loss, or microhardness.

Studies describing in vitro or in vivo caries formation or in situ studies investigating primary caries were not included in this review. Studies where caries was formed in vitro but samples were placed in in situ devices afterwards to investigate remineralization were also excluded. Studies that did not measure demineralization, but only considered outcome measures such as microtensile bond strength or biofilm composition were also excluded.

Where possible, outcome measures were translated into lesion progression rates in micrometres per day. In order to be included in the calculations for progression per day, the article had to measure caries progression according to cross-sectional microhardness or microradiography, and report lesion depth or hardness values for a composite group in enamel.

Search Strategy

The databases Medline, Cochrane, and Embase were searched for relevant articles. The Cochrane database was also searched for ongoing trials. The search strategy was composed of controlled vocabulary and free text words around the terms: "dental caries," "restorative material," "secondary caries," "in situ," and "models." Reference lists of eligible articles were hand-searched in an attempt to detect other potentially eligible studies. There was no limit as to the date or language of the articles. The exact search strategies for both Medline and Embase can be found in online supplementary Appendix 1 (see www.karger.com/doi/10.1159/000487200 for all online suppl. material).

Study Selection

All retrieved articles were stored in Endnote X7.2® software (Thomson Reuters, San Francisco, CA, USA). Duplicates were identified and excluded using the software. Two independent examiners (A.C.C.H. and N.K.K.) assessed all studies. The selected studies' titles and abstracts were carefully screened based on the inclusion criteria. If doubt existed based on an article's abstract, the full text version was reviewed. The studies considered eligible were ordered as full-text articles. In case of disagreement, a third reviewer (M.-C.D.N.J.M.H.) decided on eligibility. Inter-examiner reliability about which studies to include was determined by using Cohen's κ on the decisions made by both researchers (based on title and abstract).

Data Collection and Risk of Bias Assessment

All included articles had the following data extracted by two independent examiners (A.C.C.H. and T.T.M.): number and age of volunteers; dental status of volunteers; research conditions of the article; study design; location of the samples; method of plaque promotion; the number of weeks the devices were worn; surface lesion depth; enamel/dentin or combined samples; human or bovine dental material; the restorative materials used; fluoride in the model; sucrose-dipping conditions; whether the devices were worn during meals; gap methods and sizes; the number of drop-outs of volunteers; analysis methods; the number of samples; the results of the study; and caries lesion progression per day in the composite control group in enamel (if possible).

The quality of the studies included was assessed with a tool for assessing the risk of bias, with modifications specific for in situ studies [Centre for Reviews and Dissemination, 2008; The Cochrane Collaboration, 2011]. The risk of bias assessment was carried out by two independent examiners (A.C.C.H. and T.T.M.). We defined a low risk of bias as a low risk of bias for all key domains (a score in our tool of ≤3). Average or medium risk of bias was an increased or unclear risk of bias for ≥1 domains, and corresponded with a score of 4–6 points. A high risk of bias was an increased risk of bias for >1 domain, and corresponded with a score of ≥7 or more. In cases of disagreement on data collection or risk of bias assessment, a third researcher (N.K.K.) was consulted. The inter-examiner κ value was determined for the risk of bias scores in all the articles. Specific information about the tool used for identifying the risk of bias can be found in online supplementary Appendix 2.

Calculation of Surface Lesion Depth from Microhardness

The data necessary to estimate the lesion progression per day was always extracted from the control composite group in enamel samples, in order to compare similar groups. For articles with lesion depth as an outcome variable, the average lesion depth was extracted and divided by the number of study days.

For articles with microhardness as their outcome variable, the equation: $21.19 + 3.66 \times \sqrt{KHN}$, where KHN is the Knoop hardness number, was used to estimate volume percentage numbers at several depths, all approximately 100 μm away from the tooth/restoration interface [Kielbassa et al., 1999b]. A linear relationship was then assumed for the depth and mineral volume percentage. Microradiographic techniques assume that lesion depth is reached where the tooth material reassumes 90 or 95% of its original mineral volume [Thomas et al., 2006]; 95% of the original volume of enamel, which is 87%, is 82.6%.

The depth at which the mineral volume of the enamel sample was expected to be 82.6% was estimated from the assumed linear relationship between depth and mineral volume percentage. This depth was assumed to be the average lesion depth for this study. An example of the lesion depth estimation can be found in Figure 1.

Statistical Analysis of Studies

Descriptive statistics were used to calculate the frequencies of the factors in comparison with SPSS v22 (IBM, NY, USA). Lesion depths in the models were calculated using Microsoft Office Excel 2007 (Microsoft, Redmond, WA, USA).

Results

From the 335 titles originally selected, 31 were included in our systematic review. Details of the inclusion process can be found in Figure 2. The inter-examiner agreement for inclusion or exclusion of articles (based on title and abstract), was represented by a Cohen's κ value of 0.87.

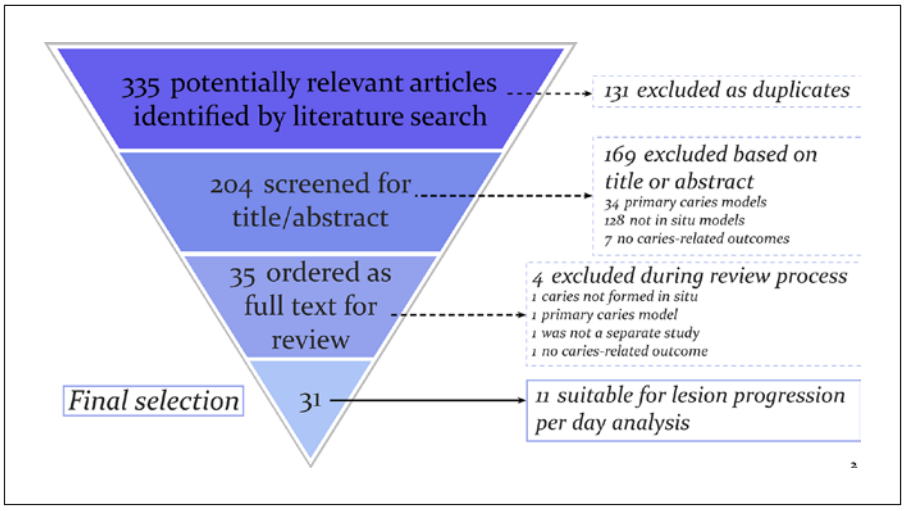


Fig. 2. Search strategy results.

Risk of bias scores per study can be found in online supplementary Appendix 3. Only 3 studies (10%) received a low risk of bias classification; 23 (74%) received an average risk of bias classification; and 5 (16%) received a high risk of bias classification. The inter-examiner-weighted κ value calculated for the risk of bias scores was 0.63.

Table 1 shows an overview of the research topics considered in the studies and how often they occurred. Table 2 shows an overview of all relevant parameters extracted from the included studies.

A split-mouth design was used in the majority of studies (74%) and a cross-over design in the rest (26%). Enamel-only samples were used in 52% of studies, 16% used dentin samples, and 32% used samples of dentin and enamel. Both human (68%) and bovine (32%) tooth materials were used. The restorative materials used varied greatly, but almost every study used at least 1 resin composite.

The in situ appliance design could be divided into 3 main groups: upper jaw appliances with samples placed in a palatal position (68%), and lower jaw appliances with samples placed either in the buccal flanges (26%) or approximally (6%, only in edentulous patients). Plaque formation on the samples was promoted by: not brushing the samples (13%), placing samples in a recessed position (19%), or, most commonly, using a mesh to cover the samples (68%). A protocol of dipping the device in sucrose to increase the cariogenic challenge was used in 90% of the studies. The remaining studies were conducted on (partially) edentulous patients, using the device as a prosthesis. A concentration of 20% sucrose was mostly used. Eight dippings a day was the most frequently used protocol (61% of those using a sucrose solution), with 4, 6, or 10 dippings a day being used occasionally.

Fluoride-containing toothpastes were used in 48% of the studies, and 42% had their volunteers use fluoride-free toothpaste. The remaining studies did not report the toothpaste used, or the fluoride in toothpaste was a research condition. Only 8 studies out of the 31 reported the fluoride concentration in the drinking water consumed by the participants.

Lesion development was measured using microradiographic techniques (TMR or transversal wavelength independent microradiography [TWIM]) in 36% of the studies, 48% used cross-sectional microhardness, and 16% used other analysis methods, such as quantitative light fluorescence or polarized light microscopy. For studies reporting >1 analysis method, the method presented as the main result was considered in this review. Eleven studies could be used to calculate or estimate average lesion progression per day (Table 3). Lesion progression varied between 1.1 and 8.8 μm/day. However, 2 main trends could be observed, with 4 studies showing a rate of ≤2 μm/day and 7 a rate of >4 μm /day. These 7 studies with a high progression rate were all palatal models, used mesh for plaque promotion, and an experiment duration of only 2 weeks.

Discussion

Parameters of in situ models used to investigate secondary caries varied greatly. Caries-related outcome measures for different dentate models were translated

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Table 1. Research topics of the studies

Research condition	*N*	Studies
Different restorative or bonding materials	21	Benelli et al., 1993 Dijkman and Arends, 1992 Kielbassa et al., 2003 Kuper et al., 2014 Melo et al., 2013 Pinto et al., 2015 van de Sande et al., 2014 Cenci et al., 2008 Jorge et al., 2015 Kirsten et al., 2013 Lennon et al., 2007 Moura et al., 2004 Pinto et al., 2009 Vasconcelos et al., 2014 da Silva et al., 2010 Kielbassa et al., 1999a Kuper et al., 2015 Melo et al., 2014 Paradella et al., 2008 Sousa et al., 2009 Wang et al., 2010
Degradation or aging of interfaces	7	Barata et al., 2012 Kuper et al., 2014 Reinke et al., 2012 de Moraes et al., 2016 Lima et al., 2009 Hara et al., 2006 Montagner et al., 2015
Er:YAG laser preparation	4	Chimello et al., 2008a, b Jorge et al., 2015 Colucci et al., 2015
Fluoride toothpaste	2	Cenci et al., 2008 de Moraes et al., 2016
Secondary lesion progression	1	Thomas et al., 2007

into values of caries progression per day, which were then compared.

In the studies considered in this review, 3 main possibilities could be identified when it came to sample placement: buccal, palatal, and approximal placements. Approximal placements were only applied in edentulous participants. This makes sense for a practical reason, since space and appliance height are necessary for these placements.

The study design that was chosen depended partially on the research question. Split-mouth designs were the most popular. Studies investigating the influence of a fluoride-containing restorative material often used a cross-over design, though not always. That carry-over does not occur between the left and right side of a palatal model seems to be an assumption shared by research communities; however, this is debatable, since the studies that we included provide insufficient evidence of this [Sousa et al., 2009; Melo et al., 2013, 2014]. More evidence of the absence of a carry-over effect in split-mouth studies needs to be gathered. Cross-over designs are definitely required when a treatment of the whole mouth is studied.

The time it takes for a clinical approximal lesion in the posterior region to progress through the enamel is estimated at 4–6 years [Fejerskov and Kidd, 2015]. The thickness of the enamel in upper and lower premolars lies, on average, between 2.3 and 2.7 mm [Vellini-Ferreira et al., 2012]. Therefore, the average clinical progression of an approximal lesion is around 0.5 mm/year. This is equivalent to 1.4 μm/day. Despite the facts that this applies to an approximal location, concerns primary caries, and has individual variability, we compared this rate with the progression rates in this review. We observed that some models, mainly with samples in the lower jaw, produced lesion progression rates close to the clinical reference. However, most studies showed rates approximately 4-fold higher, representing a somewhat "forced" caries formation. The common denominators for these models appear to be: palatal location, plaque promotion with a mesh, and a relatively short experiment duration. As such studies often have a cross-over design requiring multiple experimental periods, a high lesion progression rate may have been aimed for, in order to limit the overall study time. One study with a palatal location and mesh-covered samples showed only low caries progression rates [Benelli et al., 1993]. We speculate that this was related to the fact that they used samples with a natural surface, and not flat polished samples like in the other studies, as this will slow down initial lesion formation.

Converting cross-sectional microhardness values to mineral volume values is controversial, and the best way to do this is so far unclear [Magalhaes et al., 2009]. In general, it should be avoided where possible. In the future, it would be helpful for in situ studies to measure lesion depth microradiographically, in order to minimize doubt and simplify the comparison of different studies.

Both human and bovine tooth materials were used in the included studies. When human tooth material is unavailable or unsuitable, bovine tooth material is the most common substitute [Yassen et al., 2011]. Bovine enamel

Table 2. An overview of the included studies and their research methods

First author	Year	*N*	Age, years	Dent?	Study design	Location	Plaque+	Sucrose[a]		Time, weeks	Enamel/ dentin	Human/ bovine	Material	F–TP	F–DW, ppm	Analysis
								n	%							
Barata	2012	10	–	yes	split-mouth	palatal	mesh	8	20	4	both	human	Comp	no	0.7	Mac, PLM
Benelli	1993	10	20–25	yes	cross-over	palatal	mesh	8	20	4	enamel	human	GI, Comp	no	0.7	CSM
Cenci	2008	14	18–31	yes	cross-over	palatal	mesh	10	20	2	both	human	Comp, RMGI	RC	0.7	TMR
Chimello	2008b	13	20–35	yes	split-mouth	palatal	mesh	6	20	2	enamel	human	Comp	yes	U	PLM
Chimello	2008a	12	20–33	yes	split-mouth	palatal	mesh	6	20	2	enamel	human	Comp	yes	U	CSM
Colucci	2015	15	18–30	yes	split-mouth	palatal	mesh	6	20	2	enamel	bovine	Comp	yes	U	CSM
da Silva	2010	20	18–30	yes	split-mouth	palatal	mesh	8	20	3	both	human	Comp	no	U	CSM
de Moraes	2016	20	–	yes	cross-over	palatal	mesh	10	20	2	both	human	RMGI, Comp	RC	U	CSM
Dijkman	1992	10	32–63	no	split-mouth	buccal	RP	–	–	4	both	human	Comp, Comp with F–	no	U	TMR
Hara	2006	16	24±4	yes	cross-over	palatal	mesh	4	20	2	dentin	bovine	Comp	yes	U	TMR
Jorge	2015	20	–	yes	split-mouth	palatal	mesh	8	20	3	enamel	human	Comp, GI, RMGI	no	U	CSM
Kielbassa	1999a	11	–	yes	split-mouth	buccal	NB	var.	10	4	enamel	human	Compm, RMGI, GI		U	TMR
Kielbassa	2003	11	21–46	yes	split-mouth	buccal	RP	var.	10	4	enamel	human	Comp, Compm, GI, RMGI	no	<0.3	PLM, TMR
Kirsten	2013	15	Adult	yes	split-mouth	palatal	mesh	8	20	1	both	human	Comp, GI	yes	U	CSM, SEM
Kuper	2015	14	20–57 (30.4)	yes	split-mouth	buccal	RP	8	20	3	dentin	human	Comp, Am	yes	U	T-WIM
Kuper	2014	14	20–57 (30.4)	yes	split-mouth	buccal	RP	8	20	3	dentin	human	Comp	yes	U	T-WIM
Lennon	2007	20	18–50	yes	split-mouth	buccal	NB	5	10	4	enamel	bovine	Comp, Compm	yes	U	QLF
Lima	2009	12	20–25	yes	split-mouth	palatal	mesh	8	20	4	enamel	bovine	Comp	no	0.6–1	PLM, LM
Melo	2014	20	26.4	yes	split-mouth	palatal	mesh	8	20	2	enamel	bovine	Comp	no	U	CSM
Melo	2013	25	?	yes	split-mouth	palatal	mesh	8	20	2	enamel	bovine	Comp	no	U	TMR
Montagner	2015	14	20–57 (30.4)	yes	split-mouth	buccal	RP	8	20	3	dentin	human	Comp	yes	U	T-WIM
Moura	2004	14	59±11.2	partly	cross-over	buccal	mesh	–	–	3	both	human	Cements	yes	U	CSM
Paradella	2008	12	19–29	yes	split-mouth	palatal	RP	8	20	2	enamel	human	Comp, GI, RMGI	no	0.9	PLM, SEM
Pinto	2015	17	24–36	yes	cross-over	palatal	mesh	8	20	2	enamel dentin	bovine	Comp	yes	Yes	CSM, PLM
Pinto	2009	14	18–27	yes	cross-over	palatal	mesh	8	20	2	enamel	bovine	Comp	yes	U	CSM, PLM, SEM
Reinke	2012	10	26.9	yes	split-mouth	palatal	mesh	4	20	2	both	bovine	Comp	no	0.6–0.8	CSM
Sousa	2009	23	19–36	yes	split-mouth	palatal	mesh	8	20	2	enamel	human	Comp, Am, GI, RMGI	yes	0.7	CSM
Thomas	2007	8	<75	no	split-mouth	approx	NB	–	–	20	both	human	Com	no	No	T-WIM
van de Sande	2014	9	18–75	no	split-mouth	approx	NB	4	20	8	dentin	human	Comp, Am	yes	U	T-WIM
Vasconcelos	2014	10	24	yes	cross-over	palatal	mesh	8	20	2	enamel	human	Comp	yes	Yes	CSM
Wang	2010	10	26.3	yes	split-mouth	palatal	mesh	8	20	2	enamel	bovine	GI	no	U	CSM

Am, amalgam; CSM, cross-sectional microhardness; Comp, composite; Mac, macroscopic analysis; GI, glass ionomer; QLF, quantum light fluorescence; Compm, compomer; SEM, scanning electron microscopy; RMGI, resin-modified glass ionomer; PLM, polarized light microscopy; Dent, dentate; approx, approximal; F–TP, fluoride in toothpaste; F–DW, fluoride in drinking water; Plaque+, plaque promotion method; NB, no brushing; RP, recessed position; RC, research condition; U, unknown; var., varied.

[a] Sucrose dippings per day (*n*) and concentration of sucrose solution (%).

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Table 3. Characteristics of the studies for which it was possible to calculate or estimate the lesion progression per day

First author	Location	Time[a]	Plaque+[b]	Fluoride	Material	Sucrose[c]		Lesion progression[d]
						n	%	0 5 10
Benelli, 1993	P	4	mesh	DW	H	8	20	1.1
Thomas, 2007	A	20	NB	–	H	–	–	1.1
Dijkman, 1992	B	4	RP	–	H	–	–	1.2
Kielbassa, 2003	B	4	RP	–	H	varied	10	2.0
Chimello, 2008a	P	2	mesh	TP	H	6	20	4.3
Melo, 2013	P	2	mesh	–	B	8	20	5.1
Pinto, 2009	P	2	mesh	TP	B	8	20	5.5
Vasconcelos, 2014	P	2	mesh	TP, DW	H	8	20	6.0
Pinto, 2015	P	2	mesh	TP, DW	B	8	20	6.3
Wang, 2010	P	2	mesh	–	B	8	20	6.5
Melo, 2014	P	2	mesh	No	B	8	20	8.8

P, palatal; A, approximal; B, buccal; NB, not brushing; RP, recessed position; DW, drinking water; TP, toothpaste; H, human tooth material; B, bovine tooth material.

[a] The length of time (in weeks) that the samples were worn by the participants.

[b] The plaque promotion method.

[c] Sucrose dippings per day (*n*) and concentration of sucrose solution (%).

[d] For the control group in enamel next to a composite restoration (in μm/day).

is considered an accepted alternative to human enamel in erosion research, due to its similar characteristics and properties, even though it does not resemble human enamel in all aspects [Laurance-Young et al., 2011]. A study by Fonseca et al. [2008] concluded that bovine dentin has a significantly higher radiodensity than human dentin. Currently, the comparability of human and bovine dentin is questioned more than the comparability of human and bovine enamel. Bovine dentin has significantly fewer tubules per square millimetre than human dentin, and the tubular morphology and structure are markedly different [Lopes et al., 2009]. The use of bovine dentin, as applied in 3 studies in this systematic review, can therefore be criticized [Hara et al., 2006; Reinke et al., 2012; Pinto et al., 2015].

Lesion progression after exposure to acid gel seems faster in bovine enamel than in human enamel [Edmunds et al., 1988]. This could be a partial explanation for the high lesion progression in 5 of the studies included in this review. However, there are also studies that use human enamel with similar progression rates (Fig. 3). The answer is, therefore, not clear-cut. Unfortunately, there is also no conclusive answer from the literature when it comes to the comparison of the caries process in human and bovine tooth structures [Yassen et al., 2011].

The inter-examiner reliability for the inclusion of papers based on title and abstract, was represented by a Cohen's κ value of 0.87. This almost perfect agreement could point to the fact that the inclusion criteria were clear and applied independently by both researchers [Landis and Koch, 1977; Wijne].

The tool developed for the quality assessment of the studies was specifically tailored to recurrent problems in in situ studies. Some difficulties arose when we applied this tool, and small changes were made along the way. The difficulties can be attributed to the fact that the tool had not been previously tested. Common problems pertaining to the risk of bias to be addressed in future studies include the reporting of the fluoride circumstances for the participants. The presence and concentration of fluoride in both toothpaste and drinking water are of interest and should be mentioned.

Study participants often had previous dental knowledge and some were affiliated with the study. This problem could be addressed by blinding the volunteers to the purpose of the study and the identity of the samples. Blinding of the analyzing researcher or statistician can also reduce the risk of bias, but this was only applied in 19% of the studies reviewed. The weighted κ value of 0.63 between examiners using the risk of bias tool can be interpreted as "substantial" [Landis and Koch, 1977;

Wijne], showing that the inter-observer agreement was satisfactory. Still, when interpreting the lesion progression per day values, it is valid to exercise caution, because some of these values were estimated based only on the results reported in the articles and are rather imprecise.

Many in situ models for secondary caries research have been reported and model parameters are quite variable. Differences in the plaque promotion method and the number of sucrose dippings may play a role in the rate of lesion progression. Recommendations for future studies can be formulated, regarding the design and reporting of the studies, e.g., the full reporting of fluoride exposure and increased efforts for blinding, and also the methodology, e.g., using microradiographic methods for the analysis of lesions.

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Disclosure Statement

The authors declare no conflicts of interest with respect to the authorship and/or publication of this article.

Author Contributions

Conceived and designed the study: A.C.C.H., N.K.K., M.-C.D.N.J.M.H. Inclusion of studies: A.C.C.H., N.K.K.. Data collection: A.C.C.H., T.T.M. Wrote the paper: A.C.C.H., N.K.K., T.T.M., M.-C.D.N.J.M.H.

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